LIVING SYSTEMS
INSTRUMENTATION
www.livingsys.com • VT, USA

VIDEO DIMENSION ANALYZER

VDA-10 USER'S MANUAL

DOC-246
VDA-10 User's Manual
Rev. 1.3

Living Systems Instrumentation
www.livingsys.com

THIS PAGE INTENTIONALLY LEFT BLANK

TABLE OF CONTENTS

CHAPTER 1
Introduction

The VDA-10 Video Dimension Analyzer automatically measures the apparent wall thicknesses and the diameter of cannulated and pressurized blood vessels or other structures having distinct edges. The measurements are made by visualizing the image through a television camera attached to a microscope. Edge detection changes are continuously recorded and voltage outputs are sent to the appropriate recording device.

Lumen diameter measurements may be made in vessels that are less than 500 μM in diameter whereas outside diameter measurements may be obtained in larger vessels. The instrument operates on the relative optical density changes of wall structures at the chosen level of a pre-selected scan line. The electronic principles of the technique are described in some detail in the paper: *Mechanical behavior of pressurized in vitro prearteriolar vessels determined with a video system*. Halpern W., Osol G., Coy GS. Biomed Eng, 12:463-479, 1984. Since that time, the instrument design has been vastly improved, and renamed the VDA-10 (formally V-94), while maintaining the basic principles of operation.

Principles of Operation

In brief, a single horizontal scan line is selected using the SCAN LINE knob. This line intersects the wall and lumen of a vessel whose axis is made perpendicular to the scan line by rotating the TV camera (NTSC Video Format Compatible) attached to the microscope TV adapter. Two operator-adjustable windows are then created by the START and the WIDTH controls so that they bracket the vessel walls and are large enough to accommodate any anticipated movement of the vessel wall due to constriction or dilation of the vessel during an experiment.

The windows are made visible on the video monitor by automatic highlighting of the selected scan line. The circuit generates a linear voltage ramp starting at the beginning of the scan line. As the circuitry scans the image of the vessel, the signal voltage is modulated according to the optical density of the image. The walls are invariably denser

than either the lumen or the field outside the vessel, thereby enabling appropriate trigger circuits to detect this difference. Adjustment of the LEVEL controls sets the four points of trigger discrimination, at which time the voltage levels of the ramp signal are sampled and retained in memory. The trigger points are also highlighted on the monitor to allow the operator to visually judge that the points or measurement represent the boundaries of the vessel wall. These voltages are then properly subtracted, yielding voltage differences that are proportional to both the left and right wall thicknesses and to the lumen diameter.

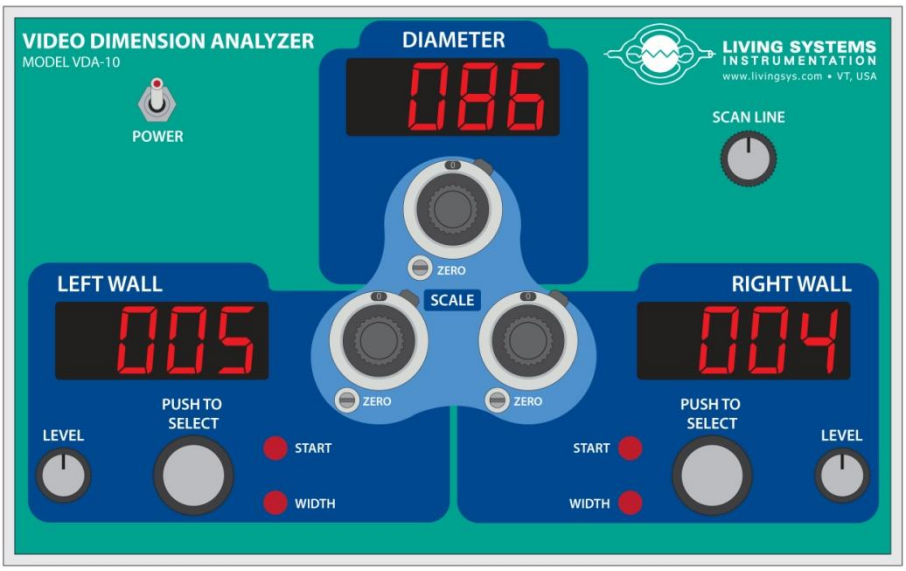
These voltages may be calibrated using a stage micrometer as described in Chapter 4. The analog output signals corresponding to LEFT WALL, RIGHT WALL and DIAMETER will be 10 mV/µM. In addition, the digital voltmeters akin to these variables read directly in microns. The resolution of these dimensional measurements is approximately 1% of the horizontal video field.

Thus, if the image of a vessel having a lumen diameter of 100 µM occupies 75% of the monitor screen width, the horizontal field is 133 µM and the resolution is about 1.3 µM. For optimum resolution it is therefore desirable to choose a microscope objective that allows the maximal outside dimension to fill a major portion of the horizontal width of the monitor screen.

CHAPTER 2
Controls and Meters

Front Panel - (Figure 2.1)

Figure 2.1 – VDA-10 Front Panel



POWER – A switch for turning the power ON and OFF. A light in the power switch handle indicates when the power is ON (see Figure 2.1).

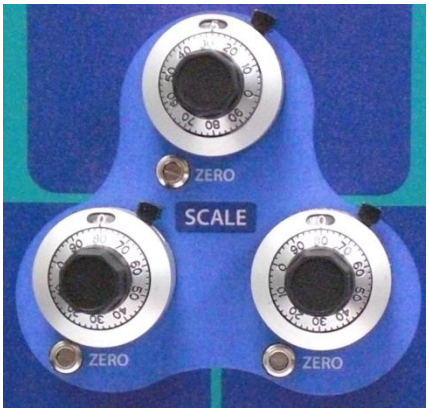
DIGITAL PANEL METERS – The three meters (DIAMETER, LEFT WALL and RIGHT WALL) have a maximum voltage reading of 19.99V and are normally calibrated to indicate dimensions directly in microns as described under the CALIBRATION section. The sampling rates of these A/D (analog-to-digital) meters is 3 samples per second. The analog output signals that are available at the BNC connections on the back panel of the VDA-10 are updated every 16.7 mSec (60 times per second, per the NTSC Video Format).

SCALE and ZERO DIALS – Each of the three panel meters has a corresponding Zero and SCALE control (see photo below). The SCALE control is equipped with a Turns Counting Knob that has an integrated locking mechanism available to the user. The

SCALE control adjusts the gain of an amplifier. Turning the SCALE control Clockwise (CW) will raise the displayed measurement value in a positive direction whereas turning the SCALE control Counter Clockwise (CCW) will lower the displayed value in a negative direction.

After successfully setting the SCALE control to the correct measurement display value, the value on the turns counting knob can be recorded for future reference. This feature can be beneficial when changing microscope objective lenses during a study. See Figure 2.2.

Figure 2.2 – VDA-10 Scale and Zero Dials



The ZERO control is located below and to the left of the SCALE control (see photo above). It has a slotted screwdriver adjustment mechanism. The ZERO control provides an offset voltage to this amplifier. Using a small screwdriver, turning the ZERO control Clockwise (CW) will raise the zero in a positive direction and Counter Clockwise (CCW) will lower the zero in a negative direction.

LEVEL – These controls adjust the triggering level of the scan line within each window to differentiate the optical density of the wall from the area external to the vessel and the optical density of the lumen from that of the wall. There is one control within each window to set the trigger point.

START & WIDTH – The START and WIDTH control pushbutton knobs allow both the positioning of its respective window to be set anywhere across the horizontal screen of

the monitor and the control of the width of its respective window. The START/WIDTH controls have an integrated pushbutton that allows the user to select the function, Start or Width, that is to be adjusted (see Figure 2.3).

Figure 2.3 – VDA-10 Start and Width Controls

When pressed and released the first time, the START/WIDTH control will "wake up" in the START control mode as indicated by the illuminated Light Emitting Diode (LED) next to the word START. When pressed and released for the second time, the START/WIDTH control will switch to the WIDTH control mode as indicated by the illuminated LED next to the word WIDTH. Each time the START/WIDTH control is pressed, it will toggle between these two functions.

After a brief period of inactivity, the START/WIDTH control will deactivate itself and both LED's will be OFF. It can easily be reawakened by pressing it again.

When the START/WIDTH control is selected for START, it will move the entire window left or right as desired without altering the size of the window.

When the START/WIDTH control is selected for WIDTH, it will control the width of its respective window by moving the right hand edge to increase or decrease the area within which measurements will be made.

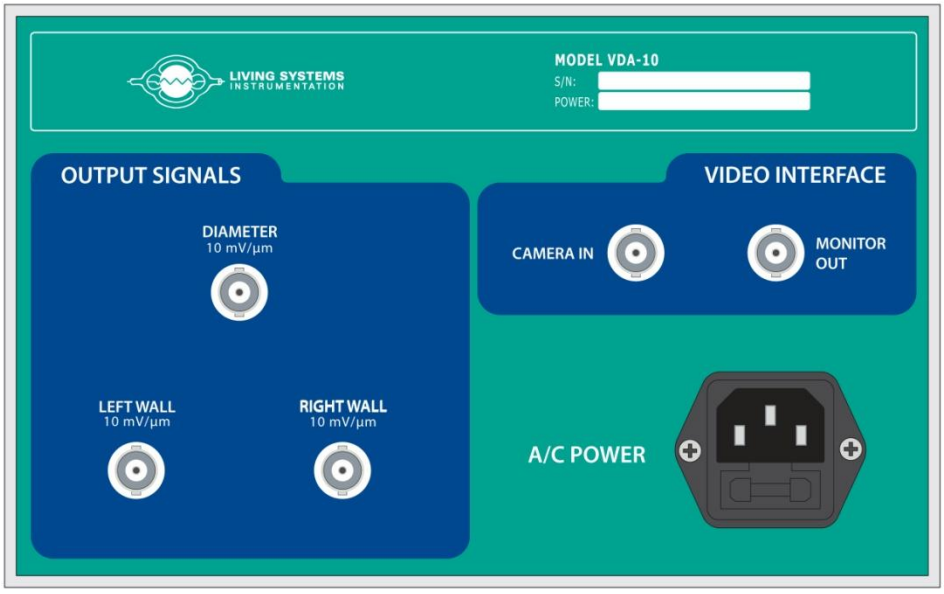
NOTE: If the user needs to reorient themselves to the relative positions of the Left Wall and Right Wall windows, they can rotate the START and WIDTH control for both

windows to the maximum counterclockwise position. This will place the minimized Left and Right Windows respectively near the left end of the scan line (*both LEVEL knobs must be fully counterclockwise – at the 9:00 o'clock position*). Then the user can begin to adjust the Start and Width position of each window of each as required.

SCAN LINE – This control selects the vertical position of the horizontal scan line to determine where vessel measurements will be made. Note that the white scan line should appear near the center of the screen with the indicator line on the Scan Line control knob pointing up – between 11:00 and 1:00 o'clock.

Rear Panel - (Figure 2.4)

Figure 2.4 – VDA-10 Rear Panel



CAMERA IN – BNC connector for 50 or 75 ohm cable from the Black and White or Color TV camera (NTSC Video Format Compatible).

MONITOR OUT – BNC connector for 50 or 75 ohm cable to the Black and White or Color TV monitor (NTSC Video Format Compatible)

DIAMETER, LEFT WALL and RIGHT WALL – BNC connectors having analog voltage outputs corresponding to the dimensions indicated on the associated panel meters. These voltages are 10 mV/□m, and are updated once every 16.7 ms – 60 times per second (per the NTSC Video Format). Thus, a 50 mV signal is equivalent to 5 □m (005 on the meter), and a 2.50 V signal represents 250 □m (250 on the meter). Each of the three signals is derived from the low impedance output of an operational amplifier. Hence, connection to a recorder or the analog-to-digital input circuit of a data acquisition system will not affect the calibrated sensitivities.

POWER – A three-pole power jack for the line cord. The operating line voltage (mains voltage) is selectable internally at time of order. It can be 115 VAC or 230 VAC at either 50 or 60 Hertz to allow the VDA-10 to be used in various countries worldwide. The voltage selected is indicated on the back panel.

MODEL and SERIAL NUMBERS – These indentify the equipment and should be referenced in communications with Living Systems.

CHAPTER 3
Connecting the VDA-10 in a System

VESSEL SYSTEM CONFIGURATION – Except for the power cords, all electrical connections are made using BNC cables. Figure 3.1 and Figure 3.2 show the use of the VDA-10 in a system consisting of the Camera, Monitor, Data acquisition, a mounted vessel in a chamber, the microscope, Flow Control Pump, Pressure Servo Control and Perfusion Pressure Monitor (with transducers).

Figure 3.1 – The VDA-10 Connected in a System

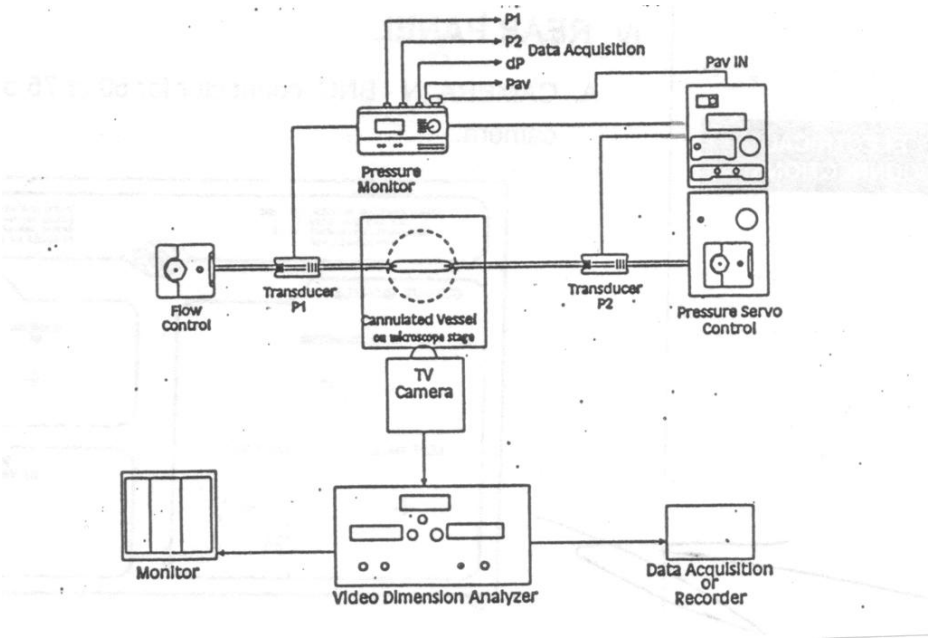
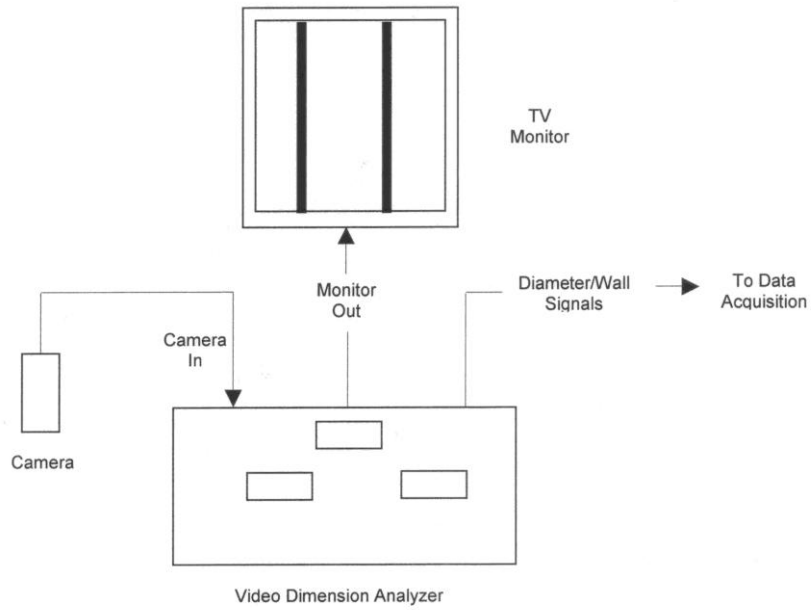


Figure 3.2 – The VDA-10 Connected in a System Closeup



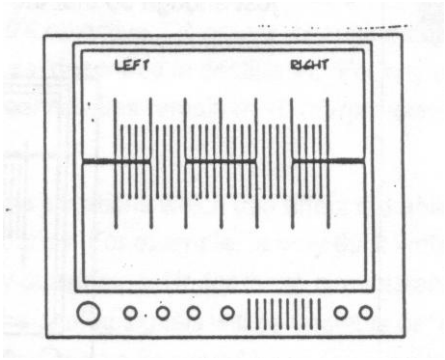
CHAPTER 4
Calibration

This procedure assures that the digital panel meters read directly in microns, and that the VDA-10 rear panel analog output signal levels are 10 mV/µM.

ADJUSTING THE WINDOWS: WALL MEASUREMENTS

1. Turn on the power of the TV camera, monitor and the VDA-10 Video Dimension Analyzer. Place a stage micrometer on the microscope stage and focus the engraved lines using a normal level of illumination so that the gradation lines are clearly seen.
2. Observe the monitor image of the stage micrometer and rotate the camera to make the long axis of the stage micrometer horizontal. If the image is uniformly gray, adjust the intensity of the microscope light source and the condenser setting to enhance the image. If the white scan line is not visible on the monitor, adjust the SCAN LINE knob until the white scan line is positioned to perpendicularly intersect the divisions of the stage micrometer.
3. Observe whether the scan line is stable. If not, adjust the illumination until stability and image clarity is achieved. Note the illumination setting of the microscope light source for future use.
4. Rotate the right and left wall LEVEL control knobs fully counterclockwise. The indicator line on the LEVEL control knob will be pointing to the left – the 9:00 o'clock position (line will be in the 9:00 position – pointing directly left) - This is called "emptying", "opening" or "unloading" the windows.
5. Locate and position the left and right windows using the respective two START and WIDTH controls. Use the stage micrometer as a guide and adjust each window to a width that is somewhat greater than the thickness of the vascular wall of your particular preparation. While doing this it is important that the left window is to the left of the right window. As shown in the following diagram, position the windows so that they are about 1/3rd and 2/3rd of the way across the monitor screen. In Figure 4.1, the windows are each exactly 50 µM wide.

Figure 4.1 – VDA-10 Wall Calibration

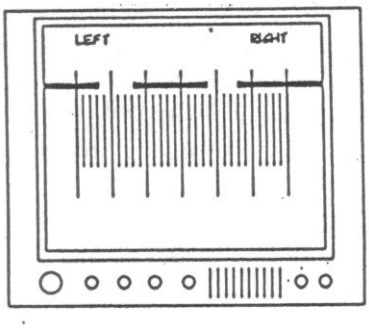


6. Turn the ZERO screw using the small potentiometer screwdriver provided (or any small screwdriver), of each window until the LEFT WALL and RIGHT WALL meters read 000.
7. Completely fill in the scan line by rotating the LEVEL controls of each window fully clockwise. You will now have a solid white line running horizontally across the monitor screen.
8. Turning only the SCALE knobs of the left and right walls, turn each Left Wall and Right Wall SCALE knobs until the meters read precisely what the known reading should be (50 µM in the above analogous drawing).
9. Re-open (empty) the windows by rotating the LEVEL control of each wall in a counterclockwise direction to ensure that the meter readings are still 000. If the readings have changed, adjust the ZERO screws of each window respectively so that 000 is again displayed on both the left and right wall meters.

DIAMETER MEASUREMENT CALIBRATION

1. Using the SCAN LINE knob, place the scan line to intersect the longer lines of the stage micrometer. Figure 4.2 illustrates this. Notice that there is some clear space on the left and right sides of each prominent line in the two windows. There must be only 1 prominent line in each window.

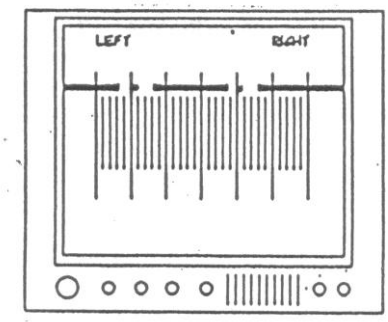
Figure 4.2 – VDA-10 Diameter Calibration



2. Change the width of each window to bracket one of the major lines of the stage micrometer (as shown in the illustration above). Again, notice that there is clear space on the left and right sides of the prominent lines in each window.
3. Turn only the ZERO screw for the diameter meter until the DIAMETER meter displays 000. (Use the zero screw potentiometer)
4. Turn only the LEVEL knob of each window in a clockwise direction – just enough so that the tiny white line (or dash) is stable. (Figure 4.3).

   NOTE: The short white line that corresponds to each wall will appear slightly skewed to the right. This is due to a timing delay within the VDA-10 and should not be considered a problem.

Figure 4.3 – VDA-10 Level Adjustments



5. Since you can easily calculate the distance in microns between these two small dashes or bracketed lines, use the SCALE potentiometer and adjust the DIAMETER panel meter to read exactly that calculated observed distance (150

μM in the illustration above). It is best to bracket lines that are as far apart as the maximum distance (diameter) you expect to find in your reactive vessel (perhaps 300 μM if you are studying a 150 μM vessel).

NOTE: If the blood vessel you are studying has a typical diameter of 150-200 μM, it is a good idea to calibrate lines that are 250 μM apart, thus allowing for constriction and dilation measurements.

6. Turn both level control knobs fully counterclockwise (to the 9:00 o'clock position) to return the readings to 000. If the zero screw needs some further adjustment, do that now. Repeat steps 1 though 6 until the readings are stable.

LINEARITY CHECK

You can now check the linearity of the diameter readings. Use just the START knob of each window. Move the window in either direction to bracket a different prominent black line and observe the resultant readings on the panel meter. Readings should be within ± 1% of the value set in Step 5 above.

RECORDER CALIBRATION

Repeating the linearity check is an easy way to calibrate a strip chart recorder or a data acquisition system. The windows may be moved to encompass whatever range you will be working in. Use the offset and sensitivity controls of the recorder. For example, at a 50 μM diameter reading and the sensitivity set, the pen may be placed at one edge of a strip chart recorder using the offset control. Then, set the windows for 250 μM and re-adjust the recorder sensitivity so that the pen is at the other edge of the paper. This will have to be repeated a few times at the high and low readings to get it right, but in later experiments, only small adjustments will need to be made since the appropriate sensitivity setting will now be established. Note that it is possible to short out your meter if the polarity of the banana plugs that go into the recording device are reversed. This will be evident if the meter reads 000 and will not follow apparent changes in diameter.

CHAPTER 5
Operational Notes

CHANGING MICROSCOPE OBJECTIVES

If experiments call for working with different-sized vessels, the objective may have to be changed to achieve optimum measurement accuracy. As an example, experiments with 150 µM diameter vessels may require a 10X objective whereas those with a 400 µM diameter vessel are better performed with a 4X objective. Obviously, changing objectives will require re-calibration as described earlier. For any objective the analog signal output sensitivities will remain at 10mV/µM once the VDA-10 is calibrated.

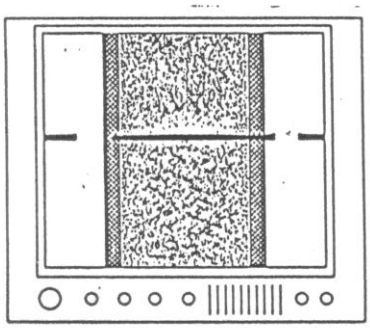
CALIBRATED INDEX CARDS

A useful tool to have on hand is an index card on which the stage micrometer gradations have been drawn. This is done by placing the card on the monitor screen on which the stage micrometer is displayed. Draw the lines and label the card in 50 µM increments. Be sure to write the identity of the objective and CCTV adapter used in the making of the card. This will enable you to use the card in situations where the edges are not quite distinct enough to make accurate diameter measurements.

IMAGE EDGE DETECTION DIFFICULTIES

There are certain situations which can affect the ability of the VDA-10 to clearly detect the wall boundaries. Two examples of this problem would be if you have a very thick walled vessel or one that is highly constricted. The digital readout and the analog signals might be unstable or "noisy" in these cases. Sometimes the image can be improved by re-focusing the microscope or adjusting the light intensity or condenser settings on the microscope. If these manipulations fail to work, the outer vessel diameter can be measured by using either of the two windows to bracket the entire vessel. Since the instrument has already been calibrated, no readjustment of the calibration settings is required. The chosen window can be positioned using the START and WIDTH controls so that the vessel fits comfortably within the empty window. Adjust the level control to achieve a stable reading. The image will appear similar to Figure 5.1.

Figure 5.1 – Stable Reading on the VDA-10



VCR RECORDING

The "live" experiment can be recorded on a video cassette recorder and used to play back through the VDA-10 to possibly make measurements at another location on the vessel, or at a later date. It is a good idea to record the image of the stage micrometer at the beginning of the recording using the same magnification as used during the experiment. By doing this you will ensure that the calibration will be on the same tape as the experiment and thus can be used for re-analysis.

CHAPTER 6

Specifications – (Table 6.1)

Table 6.1 – VDA-10 Specifications

Power – 14 W	100-120 VAC / 60 Hz	200-240 VAC / 50 Hz
Dimensional Outputs Digital Meters	Read to 1 μm	
Dimensional Outputs Analog Signal	10 mV/μm	
Measurement precision	1 – 2 μm	
Analog Signals Updated every	16.7 milliseconds	60 times per second
TV Camera and Monitor Interfaces	75 or 50 ohm; BNC connectors Compatible with the NTSC Video Format	
Weight	3.4 kg / 7.5 lbs	
Size	13.3 cm x 21.3 cm x 30.5 cm (HxWxD) 5.25 in x 8.4 in x 12 in (HxWxD)	

SAFETY NOTE

The VDA-10 must be connected to a 3-wire power outlet. It is not designed for use in an environment where explosion hazards exist.

CHAPTER 7

Contact Information

Please contact us with any questions, or if hardware issues occur.

Phone: 802-863-5547

Fax: 802-863-8057

Email: info@livingsys.com

Web: **www.livingsys.com**